THE UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER 8, CANADA

DEPARTMENT OF
BACTERIOLOGY AND IMMUNOLOGY

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Dear Dr. Lederberg,

The last time I wrote you was in the Fall of 1962, when I made some inquiry about a vacancy in your Department. Since then I have spent a year in Germany and came back only recently to Canada. Since Jan. 1st, 1964 I am appointed to the Staff of this Department. In the field of my recent interest: lysogeny in Mycobacteria I encountered some most interesting problems, which I should like to discuss with you.

I'd like to call the phenomenon which I am going to describe now: reciprocal conversion in a mycobacterial host-virus system. As you may imagine, I have no evidence of the incorporation of a homologous segment of the host genome into the phage genome, however, at present, this does not seem to be the most important question. Actually, from the two alternately possible explanations:

1. exchange of a chromosome segment between phage and host, and

2. inducing activity of the modified host genome, which in turn to conversion by the phage, "converts" the phage itself, experimental evidence or at least

my interpretation of experimental data are in support of the second mechanism. Preliminary results indicate, that profound changes found in the genetic make-up of this phage are not of the known mutational nature; neither due to induction of a carried prophage by a superinfecting phage, nor to recombination of carried and superinfecting phages.

Last year in Borstel, I isolated with Dr. Bönicke several mycobacteriophages, which were initially polyvalent, lysed several species of the genus Mycobacterium. By deadaptation through serial passages in a single host organism, we obtained hereditary stable, species-specific phages for (and within) the group of rapidly growing mycobacteria. With the exception of one phage, which is not the subject of present study, none of the phages have lost or changed their newly acquired specificity in the course of 20 passages and are still strictly monovalent.

I started my work in Vancouver by attempting to lysogenize M. smegmatis, M. phlei, M. fortuitum etc. with our „virulent"* phages. They proved unexpectedly good

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* Virulence does not refer to the origin or past history of these phages (they might have been liberated prior to their isolation from lysogenic Mycobacteria into the soil, or even be produced in the course of enrichment (soil samples with Mycobacteria); it refers only to the fact, that they lyse all studied strains of a single species which are not lysogenic for them or related other phages.

lysogenizing agents. — Lysogenic organisms were picked from the residual growth in centers of massive lysis and were purified by single colony transfers. The isolated phages*, on the other hand, were purified by single plaque transfers.

The lysogenic complexes thus established, showed with one exception, the characteristic picture known for other lysogenic systems: i.e. production of phage and immunity to homologous phage. In each instance, conversion could also be observed in as much as each lysogenic complex showed in addition to immunity against homologous wild type phage and „temperate" derivates thereof, a linked resistance to a related phage.

In the one exceptional complex, the isolated phage changed its host range very drastically in the course of its „reduction" (?) (mutation?) to prophage. It became polyvalent, somewhat differently though from its Boivin ancestor.

In order to explain the changes one might have postulated a priori:

1. A host induced modification,
2. Host range mutation
3. Induction of prophage by superinfecting phage,
4. Recombination of prophage and superinfecting phage,

* I shall try to avoid as much as possible the use of the term: temperate, since the converted phages possess not only an extended host range, but similar lytic activity to that of their wild type ancestors.

5. Recombination of phage and host genome,
6. „Conversion" of phage due to previous bacterial conversion caused by it.

Ad 1. Host induced modification could be excluded easily and quickly:
a. In serial single plaque transfers on the original host never did the new phage loose its changed host range,
b. It was in no previous contact with new host

Ad 2. If plates with same efficiency on original and new host, if it is maintained or purified on original host, the relative E.O.P. being: 1!! This excludes mutation and favors an in toto conversion of phage population derived from this lysogenic complex.

Ad 3. If it were a case of prophage induction, the host, which carries the prophage would not be attacked by homologous phage isolate (discounting the „Immune" phenomenon) However, this is not the case. Attempts to isolate phages from uninfected hosts did fail, so far.

Ad 4. Segregation into different plaque or host range types were
a) never observed on the original host, however

b. two plaque types with correlated host range differences were formed on new host. If the possibility of a second host range mutation, selectively favored by the new host, can be excluded, this then would be a segregation due to recombination. Observations show, that each type can give rise anew to the other one, too, on passage in the new host, but regains its uniformity by transfer to the original host.

c. If the two plaque types on the new host were segregant due to recombination of superinfecting phage with prophage carried by the new host, the parental segregant:
1. would not lyse the new host, which then would carry this phage in the form of prophage, and conversely
2. it should be more active against sensitive strains of same species than the other plaque type-phage.
Nevertheless, both types plate with similar efficiency on new host and relative E.O.P. of „segregants" on other strains of this species is alike 1.

d. It is true, on the other hand, that one single transfer through the new host suffice to reduce dramatically the E.O.P. of converted phage for the original strain. (This may be reminiscence of the past history of this phage, since deadaptation in Bordet followed exactly the same pattern). Thus there is a change in the relative E.O.P. as

compared to previous $1)^{6}$ 10^{-4} with one plague type and 10^{-6} or less with the other. This change is unidirectional, and original relative E.O.P. can be restituted by propagation on the original host.

5. and 6. By exclusion of the better known genetic mechanisms, we are left with the probability of a phage-host genome interaction. While there is no direct evidence for this, there are several lines of reasoning which might substantiate this claim:

1st: This complex, while reproducible, is nevertheless unique so far, since replacement of either host or phage results in the formation of the conventional lysogenic complexes only with no concomitant changes in the host range.

2nd: No further mutations occur on the original propagating host, the phage establishes itself as a "stable phage strain" for the host (with which it might or might have not exchanged a chromosomal segment). This is expressed in the uniform plaque and host range type as opposed to that on propagation in the new host.

3rd While the changes in these phages are

reproducible in case lysogeny is established with same host, they differ strikingly from related complexes. Related complexes show cross immunity and linked resistance to other phages. There is no immunity, however, against converted phages, even such complexes are lysed – and with same efficiency as their sensitive ancestors – which carry the common phage ancestor (the host being only a different strain of same species) The loss of homologs, due to which no immunity can be conferred by carried „identical" phage upon related complexes, shows the extent of change.

The entire mechanism is both phage and host specific (refering to the formation of the complex), furthermore host dependant (its stability being conserved only in the original host)

6. The frequency with which changes occur in the host range of converted phage is irreconciable with mutation or recombination. Each phage particle isolated from these lysogenic complexes has actualy a changed host range, since E. O. P new host / original host is 1, when obtained from a single plaque purified on the original host.

I am aware that these results are of preliminary nature. No further characterization of wild type phage and its derivats can be done without investigating their serological properties and immunological relationships.

changes in the host organism, on the other hand, must also very carefully be studied. Segregation into lysogenic and sensitive organisms has been already observed (I presume that the appearance of „spontaneous lysis" in lysogenic cultures was due to presence of sensitive cells in neighborhood of lysogenic organisms; frequency approx. 10^{-6}).

I don't know, if I am justified, however I have high hopes, that apart from providing an appropriate tool for the analysis of the genetic fine structure of Mycobacteria as well as their viruses, this and similar other systems might prove appropriate models to study in general the nature of virulence and temperateness and may eventually offer a new experimental approach to the further elucidation of such as yet „speculative" questions as reciprocal genetic changes (involving or not exchange of chromosomal segments) and their significance in the correlative evolution of bacteria and viruses. But this is enough from philosophy and I may be wrong.

I hope I did not inconvenience you very much with this long letter. But I rely very much on your help and criticism, for which I say you thanks in anticipation

very sincerely yours

Stephen S Yoshida

P.S. Do you think, that due to the chromosomal location of prophage, cross lysogeny is of taxonomic value? I have some data on cross lysogeny in species considered otherwise quite distantly related (not more anyhow than Salmonella and Escherichia or Shigella and Escherichia). I wonder, what the value of it was?!